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THE ESTROUS CYCLE OF THE DEER-MOUSE, *PEROMYSCUS MANICULATUS*

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THE estrous cycle has been studied very thoroughly in the house-mouse, the Norway rat, and the guinea-pig but has not been previously studied in any species of the genus *Peromyscus.* In the present investigation, the vaginal smear method was used, and histological sections were also made of the reproductive systems of several females in order to correlate the time of ovulation with the type of cells in the vaginal smears and with other changes in the reproductive organs.

MATERIALS AND METHODS

The mice used in the study of vaginal smears were 39 female progeny from a stock of the deer-mouse, *Peromyscus maniculatus bairdii,* trapped in the vicinity of Gregory, South Dakota, by H. J. Leraas and B. T. Ostenson. Virgin females of ages between 6 months and 1 year were chosen. Vaginal smears were taken with a platinum loop 3 times daily (9 A. M., 3 P. M., 9 P. M.), the intervals between examinations being 2 periods of 6 hours each and 1 period of 12 hours. With 3 examinations daily it is possible to determine the total length of the estrous cycle quite accurately, as well as the length of the various stages in the cycle.

Vaginal smears were taken on 19 mice for a period of 16 days. Because the mice became increasingly difficult to catch and handle as time went on, they were replaced by a second group of 20 mice which were likewise studied for 16 days.

The vaginal smears were spread on microscope slides and stained with haematoxylin and eosin.

In the histological study of ovulation and of changes in the reproductive system, several females of mutant strains of the same species, *P. maniculatus,* were used. Most of these animals belonged to the subspecies *gambelii.* The estrous cycles of these females were followed by means of vaginal smears, and each animal was sacrificed at a certain stage in the cycle. The ovaries, oviducts, portions of the uterine cornua, and vagina were removed, fixed in Gilson's mercuro-nitric mixture, and stained *in toto* in Mayer's carmalum. Serial sections were cut 10 microns in thickness, and no destaining was required when the material was left overnight in the carmalum stain.

Since the analysis in this study of the estrous cycle is based upon the vaginal cell content, I shall explain the criteria used in my interpretation of the various stages. Because the estrous cycle in *Peromyscus* is similar to that in other small mammals, it seems logical to follow the usual method and divide the cycle into 4 main divisions. The criteria I have used for distinguishing the stages are as follows:

Proestrum.—This begins when a marked reduction in number of leucocytes occurs accompanied by a corresponding increase in the number of epithelial cells in the vaginal smear. Long and Evans's (1922) interpretation of proestrous smears in the rat as consisting entirely of epithelial cells was not followed because of the extremely short duration of such a period in *Peromyscus* (6 hours or less). In fact quite often proestrum in *Peromyscus* is marked by the appearance of cornified cells accompanied by a gradual disappearance of leucocytes, and relatively few nucleated epithelial cells are present. Drawings of early and late proestrous stages are shown in Plate I, Figures 1 and 2.

Estrum.—An animal is considered as being in estrum when the vaginal smear consists almost entirely of cornified cells with no leucocytes present; commonly a few epithelial cells are present in early estrum. In late estrum a few dark staining nucleated epithelial cells appear among the clumped cornified cells. A typical estrous smear is shown in Plate I, Figure 3.

Metestrum.—A mouse was regarded as being in metestrum when the spindle-shaped, dark-staining, epithelial cells which appear in late estrum have almost entirely replaced the cornified cells. In early metestrum, the smear consists predominantly of these nucleated epithelial cells often in great numbers. No leucocytes are present, but a few cornified cells may persist. Soon, however, leucocytes appear and increase in number while the epithelial cells rapidly decrease in number. This stage ends when the typical diestrous condition of the smear is reached. Early and late metestral smears are featured respectively in Plate I, Figures 4 and 5.

Diestrum.—The smear in diestrum (Pl. I, Fig. 6) consists almost entirely of leucocytes with a few scattered epithelial cells. In rare cases a few cornified cells may persist from the previous stage, and quite often diestrous smears will show epithelial cells in groups of 2 or 3.

ESTROUS CYCLE AS DETERMINED BY VAGINAL SMEARS

Of the 39 female mice used in this experiment 30 passed through 1 to 4 estrous cycles during the 16-day period in which they were observed. It was possible to determine the total length of the estrous cycle only in those animals which passed through 2 or more cycles. The total lengths of 18 cycles were obtained, and these vary from 4.0 to 5.8 days, the average being 4.8 days. Only one mouse showed a cycle as short as 4.0 days, and a previous cycle of the same mouse was 5.5 days long. The limiting factor in the length of the estrous cycle is usually the duration of the diestrous period. Although very few complete cycles (including diestrum) are available, it seems quite probable that in this species, the range of the estrous cycle falls between 4 and 6 days.

The average length of proestrum, determined from 25 of these stages which began and ended at such time that they could be determined with accuracy, is 20.7 hours. Single proestrous stages vary from 15 to 30 hours, but in all but 3 cases fell within the limits of 15 to 24 hours.

The average length of 27 estrous stages was 26.2 hours. The most common duration of estrum was 24 to 27 hours. In all but 2 or 3 abnormally long periods, the individual values fell within the limits of 21 and 36 hours. These abnormally long stages, since they were few in number and obviously atypical, were not included in the average. One mouse at the end of the experiment had been in persistent estrum for 6 days and another mouse for 5 days.

OVULATION

The examination of serial sections of the ovaries and oviducts of a limited number of females indicates that ovulation takes place spontaneously near the middle or end of the cornified cell stage (estrum). The following cases are cited as evidence for this conclusion:

Female 1: Diestrum.—Three ova were present in the lower end of the oviducts. No corona cells, spindles, or polar bodies were seen, indicating that ovulation had probably occurred 3 or 4 days before, during the previous estrum. Since the ova were well preserved this stage is doubtless an early diestrum. Allen (1922) found that in the house-mouse, ova liberated in one estrous stage may often be found in the lower part of the oviducts in the following diestrous stage, and may even persist until the next proestrum, providing the diestrous stage is short.

Female 2: Diestrum.—No ova were found in the oviducts, but the presence of new *corpora lutea* in the ovaries indicates that ovulation had taken place at the previous estrum.

Females 3 and 4: Proestrum.—No ova were present in the oviducts. Large but premature follicles were present in both ovaries.

Female 5: Late estrum.—This specimen showed a late stage of estrum in the vaginal smear, which consisted entirely of large numbers of cornified cells. Ovulation had already taken place, and several ova were present in the oviducts, surrounded by large numbers of corona cells. Ovulation in this animal evidently took place earlier than in the other females studied, probably near the middle of the cornified cell stage.

Female 6: Transition stage between estrum and metestrum.—Three ova were visible in the upper ends of the oviducts surrounded by large numbers of corona cells. Appearance of the ova suggested that in this case ovulation had just taken place, toward the end of estrum.

Female 7: Early metestrum.—The vaginal smear consisted entirely of nucleated epithelial cells. Three ova surrounded by corona cells were found in the upper section of the oviducts. A spindle could be seen in 1 ovum but no polar bodies were observed. The presence of these ova in the upper ends of the oviducts indicated that ovulation probably took place near the close of the cornified cell stage.

Female 8: Late metestrum.—Two ova in 1 oviduct and 1 ovum in the other were found. The ova were not surrounded by corona cells. The second spindles and first and second polar bodies were plainly visible. The absence of corona cells indicated that these ova had been in the oviducts for several hours, but the fact that all 3 polar bodies were present shows that the ova were not disintegrating. Since metestrum lasts on the average 20.3 hours, it is logical to conclude that ovulation took place not earlier than the middle of the cornified cell stage and that the ova had been in the oviducts between 1 and 2 days.

CHANGES IN THE VAGINAL EPITHELIUM

Changes in the epithelial lining of the vagina during the estrous cycle of the deer-mouse are very similar to those described by Allen (1922) for the house-mouse.

During diestrum the epithelium of the vagina is only 5 or 6 cells deep. Leucocytes are abundant in the 1 or 2 outer cell rows but not in the deep rows. Leucocytes are numerous in the vaginal lumen, and there are also a few epithelial cells. The lumen is folded and collapsed. The epithelium has no basement membrane.

In proestrum, the outer 4 or 5 cell rows of the vaginal epithelium stain very lightly, and this outside layer may separate from the rest of the epithelium and peel off into the lumen. Beneath this outside layer is a thin dark-staining granular strip and directly beneath this are 10 to 13 rows of healthy looking epithelial cells.

During estrum the outer 4 or 5 light-staining cell rows of the epithelium have disappeared and the underlying granular layer, which is now cornified 10 to 12 cells thick, is being shed. Beneath the cornified layer still remain 10 to 13 rows of normal epithelial cells.

In early metestrum the semi-collapsed lumen of the vagina contains nothing but nucleated epithelial cells. The cornified layer of the epithelium has disappeared, and the epithelium is 9 to 12 cells thick. Leucocytes are absent. In late metestrum both epithelial cells and leucocytes are found in the lumen, the epithelium is about 7 cells thick; the outer 3 or 4 rows are heavily infested with leucocytes.

OBSERVATIONS ON THE OVARIES, OVIDUCTS, AND UTERUS

No attempt was made in this study to count numbers of *corpora lutea* or to distinguish one set from another except in a general way. It was noted, however, that in every case there were several sets of *corpora lutea* easily distinguishable in appearance, size, and position. Ripe follicles were present in the ovaries only during early estrum. Small immature follicles were seen during metestrum and diestrum, while large but premature follicles were found in proestrum. The ovaries are larger at proestrum and estrum than at metestrum and diestrum.

The oviducts of the deer-mouse are very much convoluted, as in the house-mouse and Norway rat.

The uterus is greatly distended and thin-walled during proestrum and part of estrum and in appearance in cross-section (cornua) is very similar to that of the house-mouse. During metestrum and diestrum the lumen is much smaller and the walls thicker than in proestrum and estrum.

A more complete histological description of the reproductive system of *Peromyscus* is deemed unnecessary because it would only repeat that given by Allen (1922) for the house-mouse.

DISCUSSION

The estrous cycle of 4.8 days in the deer-mouse compares very closely in duration with that of the house-mouse and of the Norway rat. According to Allen (1922), the cycle in the house-mouse is 4 to 6 days in length. Togari (1927) found that the majority of the cycle durations studied by him were 5 days. Parkes (1928) in observations on 1000 cycles reports the length as 6.2 days. Claudberg (1931) regards the cycle in the white house-mouse as 9 to 10 days long, but in his calculation the *corpora lutea* phase following ovulation is included. Long and Evans (1922) found the estrous cycle in the Norway rat to be most commonly about 4 days in length.

A proestrous stage during which smears consist entirely of epithelial cells does not last much more than 6 hours in *Peromyscus* and is often obscure and not always observed when smears are taken at 6 hour intervals. Allen (1922) reports that this stage often lasts for less than a day in the house-mouse, and Long and Evans (1922) give its duration in the Norway rat as 12 hours. A proestrum characterized by gradual disappearance of leucocytes accompanied by increasing numbers of epithelial cells and varying numbers of cornified cells covers a period of about 20.7 hours in the deer-mouse. The cornified cell stage or estrum in the deer-mouse lasts 26.2 hours, and this agrees almost exactly with the same period in the Norway rat (27 hours). Allen (1922) states that estrum in the house-mouse usually lasts 1 or 2 days, which is about the same duration as in the deer-mouse. In the definition of stages given earlier in this paper, it was mentioned that early metestral smears in *Peromyscus* consisted almost entirely of nucleated epithelial cells. This particular stage lasts for about 12 hours and according to Selle (1922) occurs regularly in the guinea-pig, but is seen only occasionally in the rat (Long and Evans, 1922). Allen (1922) does not mention such a stage in the house-mouse.

Apparently, in both the Norway rat and the house-mouse, metestrum usually begins with an infiltration of leucocytes among the clumps of cornified cells, before the cornified layer of the vagina has been completely shed. In the guinea-pig, deer-mouse, and cotton-rat, however, leucocytes do not attack the vaginal lining until later, after the cornified layer has disappeared entirely and several rows of the underlying nucleated epithelial cells have been sloughed into the vaginal lumen.

A typical diestrous smear is not found in *Peromyscus* until about 20.3 hours, on the average, after the close of estrum, while Long and Evans give the length of metestrum in the rat as 6 hours.

The estrous cycle of the cotton-rat (Clark, in press), as determined by vaginal cell content, differs from that of *Peromyscus* only in the lengths of the various stages, being over 8 days in length while the cycle of the deer-mouse is only 4.8 days long. Estrum and diestrum in the cotton-rat are much longer than in the deer-mouse.

Parkes (1927) finds that in the house-mouse, proestrum and estrum together last 2.4 days, while metestrum combined with diestrum endures for 3.7 days. The same periods in *Peromyscus* are shorter, being 1.9 and 2.9 days, respectively.

SUMMARY

Vaginal smears taken 3 times daily on 39 females of the deer-mouse, *Peromyscus maniculatus,* indicate that the length of the estrous cycle is 4 to 6 days. This result compares quite closely with the length of the estrous cycle in the Norway rat and the house-mouse.

The average lengths of the various stages in the estrous cycle of the deer-mouse were found to be: proestrum, 20.7 hours; estrum, 26.2 hours; metestrum, 20.3 hours; diestrum, 49.9 hours; total cycle, 4.8 days. The cornified cell stage (26.2 hours), or estrum, compares very closely in length with the same period in the Norway rat (27 hours) and is very nearly the same length as in the house-mouse (1 or 2 days).

Histological sections of the ovaries and oviducts of the deer-mouse show that ovulation occurs spontaneously during the middle or last part of the cornified cell stage (estrum).

The ovaries, oviducts, uterine cornua, and vagina are structurally very similar to those of the house-mouse.

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PLATE I

Vaginal smears of the deer-mouse, *Peromyscus maniculatus*, showing the types of cells found in various stages of the estrous cycle. ×150.

FIG. 1. Proestrum (early).—The smear shows a larger number of nucleated epithelial cells than are found in diestrum, with a corresponding lesser number of leucocytes. A few cornified cells may occur.

FIG. 2. Proestrum (late).—The smear consists mostly of nucleated epithelial cells. Leucocytes are rarely present in late proestrum, but cornified cells are common.

FIG. 3. Estrum.—The smear consists entirely of cornified cells.

FIG. 4. Metestrum (early).—In *Peromyscus* as in the guinea-pig early metestrous smears consist entirely of nucleated epithelial cells.

FIG. 5. Metestrum (late).—Showing infiltration of leucocytes among the nucleated epithelial cells.

FIG. 6. Diestrum.—A typical diestrous smear consisting primarily of leucocytes with a few scattered epithelial cells.

PLATE I